NEW

Luminex® Histone H3 PTM Multiplex Assays

bead-based multiplex assay system for studying histone h3 post-translational modifications

Epigenetic mechanisms including DNA methylation, histone modification and nucleosome positioning comprise the non-genetic memory of a cell. Epigenetic modifications alter the dynamics of gene expression by causing changes in chromatin structure that influence the accessibility of *cis*-acting DNA elements to *trans*-acting regulatory factors. Because epigenetic variation is a critical determinant of cell lineage commitment, phenotypic diversity and disease susceptibility, advances in technologies for mapping and characterization of epigenetic events are highly warranted. Active Motif has partnered with Luminex®, the industry leader for multiplexing, to develop the Histone H3 PTM Multiplex Assay, the first commercially available assay for multiplexing analysis of histone modifications, that is designed for use with either the MAGPIX®, Luminex® 200™ or FLEXMAP 3D® instruments. This unique assay offers researchers the ability to gather more information from smaller sample amounts, in less time and at a lower cost than afforded by traditional methods for studying histone PTMs.

Why are Histone Modifications Important?

Histones are responsible for packaging DNA into nucleosomes, the basic structural unit of chromatin. They are subject to a variety of post-translational modifications (PTMs), including phosphorylation, acetylation, and methylation, that function to regulate gene expression and chromatin structure. The importance of studying histone modifications has implications for human health as overwhelming evidence exists correlating abnormalities in histone modifications to various human pathologies. Subsequently, histone PTMs are targets for various areas of research, including as biomarkers to predict cancer prognosis (Table 1), as markers of active (H3K4me3) or repressed (H3K9me3 and H3K27me3) chromatin, and in studies of cell signaling response.

	Histone H3					Histone H4				
Tumor Type	Multi-Kac	K4me1-3*	K9ac	K9me1-3*	K18ac	Multi-Kac	R3me2	K12ac	K16ac	K20me3
Breast		●	●		●		●	●	●	●
Colorectal									●	●
Esophageal					●		●			
Hematological									●	●
Kidney	●	●		●	●	●				
Lung		●			●					
Pancreatic		●		●	●					
Prostate		●	●*	●	●	●				●

*Recurrence predictor in high grade prostate carcinoma.

Table 1: Histone PTMs exhibit altered levels in neoplastic tissue.
The table above shows the correlation between global histone modification profiles and the overall survival prognosis for specific tumor types.

Relative to DNA methylation, far less is known about the biological significance of histone PTMs, mostly due to limitations in available technologies. Current methods to study histone PTMs include Western blot, immunostaining and genome-wide mapping. However, these methods are time-consuming, lack high throughput capability and can be costly when interrogating more than one histone PTM target. To address these limitations, Active Motif has partnered with Luminex, the industry leader in multiplexing technology, to develop the new **Histone H3 PTM Multiplex Assay**, the first multiplex epigenetic assay for the study of histone modifications. The highly sensitive assay enables high throughput processing using low (nanogram) sample amounts to interrogate multiple histone PTMs within a single well. The assay format also offers the ability to normalize PTM data against total histone H3 values to evaluate relative histone PTM levels when comparing different samples or treatment conditions.

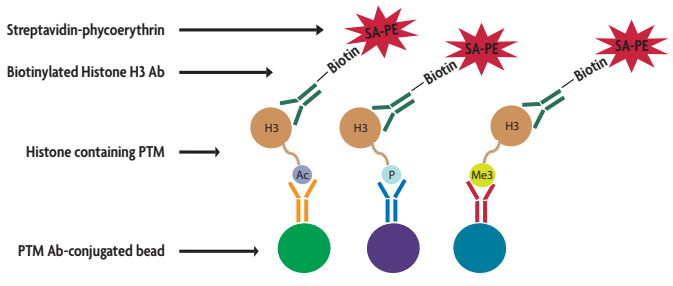

Figure 1: Schematic of the Histone H3 PTM Multiplex assay.

How Does the Assay Work?

The Histone H3 PTM Multiplex Assay works as a solution-based suspension sandwich ELISA to interrogate the levels of histone modifications from acid extracted cell lysates or purified histones. Histones are captured using fluorescent labeled magnetic beads that have been conjugated to antibodies specific for the histone H3 PTM target. A biotinylated antibody against the C-terminus of Histone H3 is then added to bind the captured histone to form a "sandwich". Next, streptavidin-phycoerythrin is added to bind the biotinylated antibody and provide a readout signal to measure antibody binding events (Figure 1, opposite page).

Because the fluorescent signal is unique to each antibody-conjugated bead set, beads corresponding to multiple analytes can be multiplexed within the same sample (Figure 2). Inside the Luminex instrument, the identity of each bead (and it's corresponding histone PTM) is deciphered based on its emitted fluorescent signal. A second light source is then applied to the same bead to determine the magnitude of the streptavidin-phycoerythrin signal. The Luminex xPONENT® software program provides a real-time readout of signal as median fluorescent intensity (MFI) to determine PTM levels. By including the Histone H3 Total bead set in the assay, values across samples can be normalized to total histone H3 levels to determine the relative amounts of each histone modification.

What's in the Box?

A complete assay requires the purchase of both the **Histone H3 PTM Multiplex Kit** and the **Ab-conjugated bead(s)** of interest. To learn more about our Luminex assays, please visit us at www.activemotif.com/luminex.

For improved sensitivity, purify your histone samples using our **Histone Purification Kits** (Figure 3).

Histone H3 PTM Multiplex Advantages

- **Interrogate up to 13 PTMs in a single well**
- **Evaluate relative PTM levels**
- **Higher throughput processing than WB**
- **Use nanogram quantities to study multiple PTMs**
- **Ideal for screening variable treatments or conditions**
- **High specificity & lot-to-lot consistency**
- **Reduction in time, cost & labor**
- **3-hour assay time**

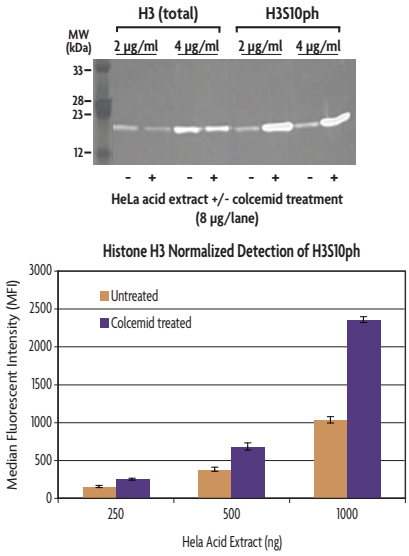

Figure 2: Comparison of H3S10ph data from a Western blot or Histone H3 PTM Assay. Total histone H3 and H3S10ph levels from untreated or colcemid-treated HeLa acid extract were evaluated by Western blot (top image) or a 2-plex Histone H3 PTM Assay using Total H3 and H3S10ph beads (bottom image). The Western is limited in throughput, requires microgram sample amounts and does not allow multiplexing or normalization of PTM values. In contrast, the Histone H3 PTM Assay enables multiplexing using only nanogram sample amounts and allows higher throughput for replicate analysis. Data normalization against total H3 values reveals relative changes in H3S10ph levels across samples.

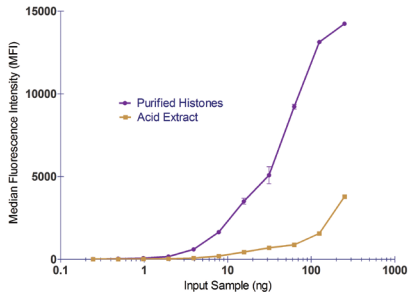

Figure 3: Histone purification improves Histone H3 PTM Assay sensitivity. A lower limit of detection is observed for the H3K27me3 assay using histones purified (purple) with AM's Histone Purification Mini Kit (Catalog No. 40025) vs crude acid extract (copper).

Product	Format	Catalog No.
Histone H3 PTM Multiplex Kit	96 rxns	33115
Histone H3 Total Ab-conjugated beads	48 rxns	33116
Histone H3K9ac Ab-conjugated beads	48 rxns	33117
Histone H3K9me1 Ab-conjugated beads	48 rxns	33118
Histone H3K9me2 Ab-conjugated beads	48 rxns	33119
Histone H3K9me3 Ab-conjugated beads	48 rxns	33120
Histone H3K4me3 Ab-conjugated beads	48 rxns	33121
Histone H3S10ph Ab-conjugated beads	48 rxns	33122
Histone H3 pan-acetyl Ab-conjugated beads	48 rxns	33123
Histone H3K27me2 Ab-conjugated beads	48 rxns	33124
Histone H3K27me3 Ab-conjugated beads	48 rxns	33125
Histone H3K56ac Ab-conjugated beads	48 rxns	33126
Histone H3K27ac Ab-conjugated beads	48 rxns	33127
Histone H3T11ph Ab-conjugated beads	48 rxns	33128

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